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CLINICAL CHEMISTRY

Simplified Methods of Collecting and Processing Whole Blood for Quantitation of Plasma Catecholamines

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A more liberal and less constrained handling of plasma norepinephrine (NE) and epinephrine (EPI) samples simplifies analysis and improves laboratory efficiency. We tested their stability in heparin or EDTA plasmas alone or combined with the antioxidant glutathione at 24°C or following long-term storage at −70°C. NE and EPI measured by high-pressure liquid chromatography (HPLC) with electrochemical detection are stable in heparinized plasma for 24 hours at 24°C (<6% degradation / d). In EDTA plasma, NE and EPI levels decrease less than 10% after 6 hours at 24°C but decay by 50% between 6 and 24 hours. At −70°C, NE and EPI in heparin or EDTA plasma are stable for 8 months, and the addition of antioxidant has no effect. Whole blood anticoagulated with heparin or EDTA does not alter plasma NE over the initial 6 hours at 24°C. We conclude that simple heparinization of human venous blood provides optimal conditions for quantitation of the in vivo concentration of plasma NE and EPI.

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The opinions expressed herein are those of the authors and are not to be construed as reflecting the views of the Navy Department, Naval Service at large, or the Department of Defense.

Plasma catecholamine measurements are important in the diagnosis and management of nervous system related disorders,[1,2] hypertension,[3,4] pheochromocytoma,[5,6] and to assess patient resuscitation following shock and or trauma.[7,8] Due to the chemical structure of the catechol ring and its susceptibility to spontaneous oxidation in buffered solutions, catecholamine stability has been of particular concern when determining quantitative levels.[7] Quantitative analysis has been achieved by fluorometric techniques,[9] radioenzymatic assays,[10,11] and high-pressure liquid chromatography (HPLC).[12,13] HPLC with electrochemical detection has shown the least interlaboratory variability when compared to other analytical techniques.[14] Different methods of blood collection including the choice of anticoagulant, the addition of antioxidants, and various sample processing techniques have also been reported.[10,15,16]

The need to rapidly process plasma catecholamines to prevent their degradation has limited their use in the clinic, especially when attempting a comprehensive analysis of critical care medicine.[17] This work was undertaken to characterize further the effects of two common anticoagulants (heparin and EDTA) and the antioxidant glutathione (GSH/EGTA) as a preservative on plasma catecholamine stability. We analyzed NE and EPI stability at room temperature (24°C) and −70°C. In addition, we measured the changes in NE and EPI levels after delaying plasma separation from whole blood, which may occur with field studies. HPLC with electrochemical detection, which affords increased sensitivity compared with other detection methods, was used to quantitate catecholamine levels. We designed these studies to establish reasonable procedures for the management of routine clinical and research laboratory samples, including samples collected in field studies conducted at some distance from the laboratory. Realistic time limitations and choice of anticoagulant for quantitating NE and EPI from whole blood and plasma are presented.

Materials and Methods

Sample Collection

Blood samples were collected from nonfasted healthy women and men, aged 25 to 55 years. Venous blood (80 mL) was drawn into tubes containing either sodium EDTA (4 mmol/L, final concentration) or heparin (14.3 USP units/mL) as anticoagulant. All blood samples except those to be used for whole-blood degradation studies were centrifuged and aliquoted within 1 hour of collection.

Blood was centrifuged at 3,000 rpm (1,000×g) for 10 minutes at 4°C in a Sorvall RC-3 centrifuge[a] to pellet cells and platelets. Plasma was pooled, placed on

ice, and aliquoted (1 mL) into 1.5-mL microfuge tubes with or without the addition of a EGTA (8 mmol/L, final concentration) and glutathione (reduced form, 6.5 mmol/L, final concentration) as antioxidant preservatives.[18,19] Plasma samples were either frozen immediately at −70°C (as controls, t = 0 minutes) for analysis after long-term storage, or incubated between 5 minutes and 24 hours at 24°C to measure decay rates. Plasma for the whole-blood studies was serially analyzed following continuous, gentle rotation at 24°C for 1, 6, 24, and 48 hours prior to removal of plasma and was stored at −70°C.

A single prospective analysis was conducted with the same pooled samples of plasma. Plasma for room temperature and long-term (−70°C) degradation analysis was pooled from a minimum of six individuals. The subject pools were different for the various experiments. This accounts for the differences in reported control values.

Extraction and Quantitation of Catecholamines

Plasma samples were extracted as originally described by Anton and Sayre.[20] In brief, alumina adsorption of catecholamines from plasma was complete by 30 minutes at room temperature. Following thorough washing of the adsorbed alumina, desorption was complete by addition of 100 mL of acetic acid containing 0.05% EDTA and 0.1% sodium disulfite. 3,4-Dihydroxybenzylamine (DHBA) was used as an internal standard to quantitate the catecholamines.[13] The extraction efficiency of NE, EPI, and DHBA was routinely 66% to 70% based on the extraction of known standards. After extraction, samples were immediately assayed by HPLC using a Waters 460 electrochemical detector[b] interfaced with a Digital 380 computer utilizing Waters Expert Chromatography software (Version 5.2)[b]. The extraction and HPLC methods allow for reliable detection of NE and EPI if values are greater than 20 pg/mL of plasma. The intra-assay coefficient of variation was 5% for NE and 8% for EPI. Assays were run in duplicate and data were expressed as the mean pg/mL ± SE. The conversion factor for expressing NE as nmol/L is 0.00591 and 5.458 for expressing EPI in pmol/L. Statistical differences were determined by ANOVA with Duncan's test between means for repeated measures[d].

Table I: Changes in the Concentration of Plasma Norepinephrine (NE) and Epinephrine (EPI) (±SE) Following Incubation at Room Temperature (24°C)

Plasma	GSH/EGTA*	Concentration, pg/mL			Decay Rate†	
		0 h	6 h	24 h	pg/24 h	(%/24 h)
Heparin						
NE	−	335±8	324±2	326±7	6±5	(2.7)
	+	299±2	285±0	282±9	14±4	(5.7)
EPI	−	43±0.8	43±0.4	42±0	0.3±1.9	(0.7)
	+	37±0	33±2	36±0.4	1.7±1.8	(4.6)
EDTA						
NE	−	333±24	329±7	165±5	180±41	(54.1)
	+	377±6	359±18	157±8	239±39	(63.4)
EPI‡	−	498±153	503±93	271±4	239±73	(48.0)
	+	452±4	447±17	236±7	231±52	(51.1)

Minus signs indicate plasma without preservative; plus signs, plasma with reduced glutathione (GSH) (6.5 mmol/L) and EGTA (8 mmol/L) as a preservative.
†Plasma incubated at 24°C was serially sampled over 24 hours. NE and EPI were quantitated by HPLC in pg/mL. Decay rates were determined by linear-regression analysis.
‡Plasma aliquots were supplemented with 440 pg of EPI.

Table II: Changes in Norepinephrine (NE) in Whole Blood Incubated at Room Temperature (24°C)

Time, h	Heparin, pg/mL	EDTA, pg/mL
0	435	394
1	404	392
6	435	409
24	158	256
48	106	120
Decay rate, pg/h*	7.43±1.58	6.03±0.59

**Decay rates for the 48-hour period were determined by linear-regression analysis. No significant change was observed in the NE concentration of EDTA or heparinized plasma for 0 to 6 hours.*

Results

Degradation of NE and EPI in Plasma (Heparinized Blood)

Plasma NE and EPI levels from heparinized blood were relatively stable after 24 hours at 24°C (Table I). Without preservative, NE and EPI decreased by only 6 ± 5 and 0.3 ± 1.9 pg in 24 hours, respectively. The addition of preservative did not significantly alter the degradation of NE and EPI which were 14 ± 4 and 1.7 ± 1.8 pg, respectively, in 24 hours. These decay rates are not concentration dependent, since there was no change in the degradation rate after 24 hours at 24°C when plasma NE and EPI was increased to 1,000 and 600 pg/mL, respectively, by addition of exogenous catecholamine (data not shown).

Degradation of NE and EPI in Plasma (EDTA Blood)

Plasma NE in whole blood collected with EDTA was stable for up to 6 hours at 24°C with or without the addition of preservative (Table I). However, both NE and EPI rapidly decayed following incubation for greater than 6 hours at 24°C. NE decreased 50% and 59% in the absence or presence of preservative, respectively, after 24 hours at 24°C (Table I). When compared with heparinized plasma, the NE decay rate in EDTA plasma was 30-fold greater in the absence of preservative and 17-fold greater in the presence of preservative (Table I). EPI degraded to 45% and 48% of the control value in the absence and presence of preservative, respectively, when compared to

a negligible loss in heparinized plasma (Table I). Since control values of EPI measured in the EDTA plasma were below the level of detection (<20 pg/mL), exogenous EPI (440 pg/mL) was added to the pooled samples prior to incubation.

Degradation of NE in Whole Blood

NE values in whole blood anticoagulated with heparin or EDTA are stable up to 6 hours at 24°C (Table II). After delaying plasma separation between 6 and 48 hours, NE values are lower than those of controls. Decay rates of 7.34 ± 1.58 and 6.03 ± 0.59 pg/h in heparin or EDTA whole blood, respectively, were similar. However, increasing the time to 72 and 96 hours before heparinized plasma is separated resulted in a further decline in NE to less than 15% of control. EPI was below the level of detection possible with HPLC.

Plasma NE and EPI Stability During Long-term Storage

NE and EPI plasma values were stable when stored at −70°C for up to 228 days with heparin (Fig 1) and 205 days with EDTA (Fig 2) as the anticoagulant. No advantages were noted with the addition of GSH/EGTA as a preservative. There was no statistical difference in the decay rates (pg/d) of NE and EPI between heparin and EDTA plasma (Table III).

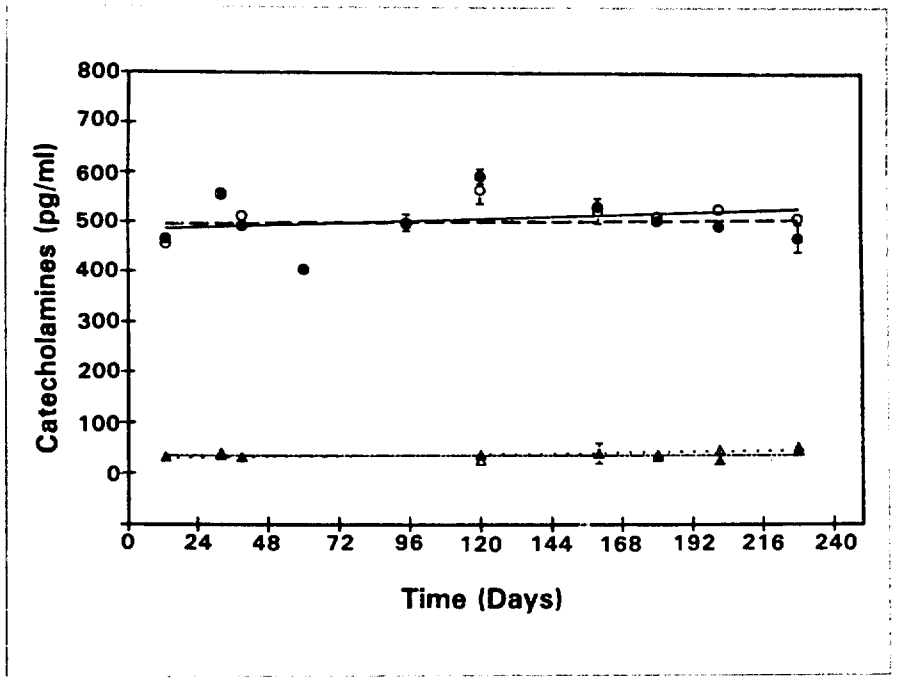

Fig 1. Plasma norepinephrine (NE) and epinephrine (EPI) levels from heparinized blood following long-term storage at −70°C with and without reduced glutathione and EGTA (GSH/EGTA) as preservative. NE without GSH/EGTA (open circles); NE with GSH/EGTA (closed circles); EPI without GSH/EGTA (open triangles); and EPI with GSH/EGTA (closed triangles).

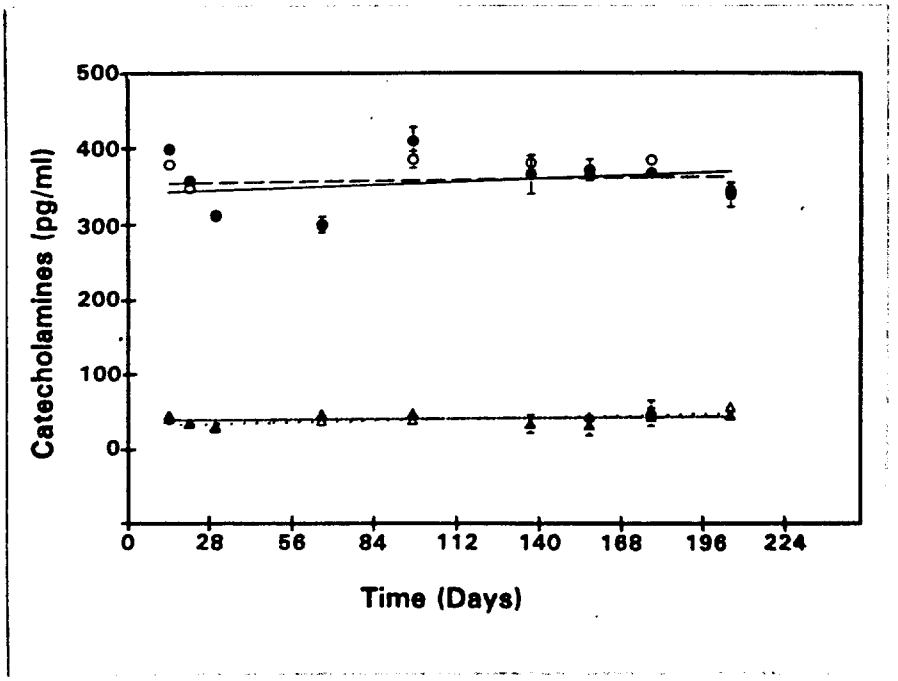

Fig 2. Plasma norepinephrine (NE) and epinephrine (EPI) levels from EDTA-treated blood with and without the addition of reduced glutathione and EGTA (GSH/EGTA) as a preservative following long-term storage at −70°C. NE without GSH/EGTA (open circles); NE with GSH/EGTA (closed circles); EPI without GSH/EGTA (open triangles); and EPI with GSH/EGTA (closed triangles).

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Our results support the previous findings that catecholamine analysis does not require elaborate collection, processing, and storage procedures. We have, however, extended these findings with a comprehensive comparison of the stabilizing effect of preservatives added to both EDTA and heparin plasma and contrasted these with the effects of celluiar elements of whole blood on plasma catecholamine values. Pettersson et al[15] previously reported catecholamines were stable in plasma from heparinized blood for 22 hours at room temperature in the absence of thiols or antioxidants. De Vera et al[21] also found no degradation of NE in heparinized plasma without addition of antioxidants for 10 hours at 37°C. Weir et al[16] showed no effect on plasma catecholamines from EDTA anticoagulated blood after 3 hours at room temperature. We find no significant change of NE or EPI values in heparinized plasma samples for up to 24 hours after separation. In

Table III: Decay Rates of Norepinephrine (NE) and Epinephrine (EPI) from Heparinized and EDTA Blood Following Long-term Storage at −70°C

Plasma	GSH/EGTA*	Decay Rate, pg/d ± SE†	n
Heparin			
NE	−	0.17 ± 0.20	10
	+	0.03 ± 0.24	10
EPI	−	0.07 ± 0.01	8
	+	0.03 ± 0.03	8
EDTA			
NE	−	0.14 ± 0.16	9
	+	0.05 ± 0.18	9
EPI	−	0.07 ± 0.03	9
	+	0.02 ± 0.04	9

*Minus signs indicate plasma without 6.5 mmol/L GSH and 8 mmol/L EGTA; plus signs, plasma with 6.5 mmol/L GSH and 8 mmol/L EGTA.
†Decay rates were determined by linear-regression analysis.

contrast, NE and EPI concentrations in EDTA anticoagulated blood were stable for 6 hours at 24°C but decreased significantly by 24 hours. EDTA plasma kept at 24°C for longer than 6 hours, a likely consequence of freezer malfunction or long-distance transport, will show markedly reduced plasma NE and EPI values when assayed with our HPLC system.

NE and EPI are rapidly degraded when stored in Tris-HCL (pH 7.4) buffer in the absence of exogenously added stabilizing agents.[15] However, since plasma catecholamines are relatively stable,[15,16] the presence of endogenous plasma antioxidants may be inferred. Therefore, adding preservatives prior to storage of plasma is not required if EDTA plasma samples are stored frozen at −70°C within 6 hours or if heparinized plasma is frozen within 24 hours. Furthermore, we show that NE and EPI values from plasma anticoagulated with either heparin or EDTA and stored at −70°C are stable for more than 200 days without the addition of a preservative.

Previous reports of delayed plasma separation from whole blood for up to 3 hours showed no change in catecholamine levels.[15,16] Our data extend this 3-hour limit to 6 hours and expand the finding to include either heparin or EDTA as the anticoagulant. We suggest collecting clinical and field blood samples for plasma NE and EPI analysis with heparin as the anticoagulant. This technique allows for long-distance transport of whole blood (6 hours) or lengthy delays after plasma separation (24 hours) without significant in vitro degradation.

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